The effect of retinal GABA Depletion by Allylglycine on mouse retinal ganglion cell responses to light

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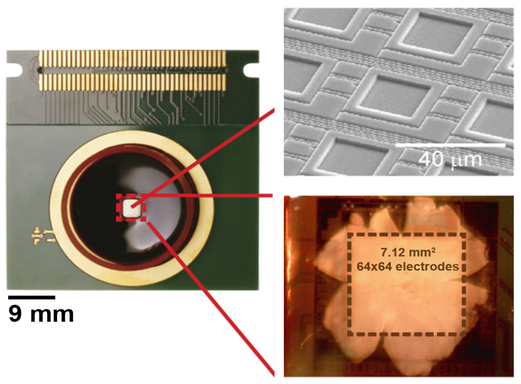
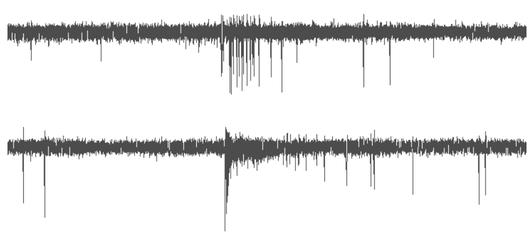
Introduction

The inhibitory neurotransmitter GABA (γ-aminobutyric acid) is metabolized by glutamic acid decarboxylase (GAD) which exists in two isoforms in the mature CNS, GAD65 and GAD67. Allylglycine, a glycine derivative, is a nonspecific inhibitor of both GAD isoforms. Prolonged exposure to allylgycine can therefore deplete the tissue of endogenous GABA over time (Orlowski et al, 1977; Chabrol et al., 2012). Here we applied Allylglycine (ALLYL) in vitro over several hours to gradually deplete GABA in the adult mouse retina and compared the effects of GABA depletion on retinal ganglion cells (RGCs) receptive fields with those obtained by simultaneously blocking all GABAergic receptors (type A, B and C).

Methods

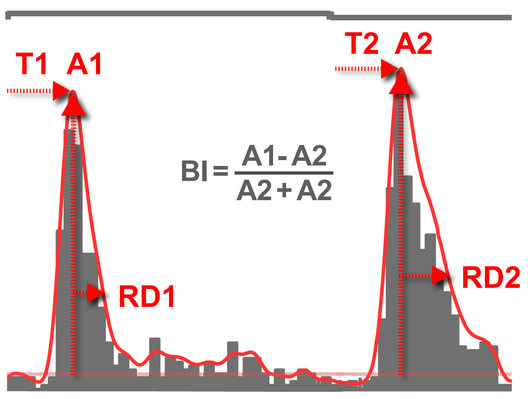


Active Pixel Sensor MEA Chip featuring 4,096 electrodes (42 µm spacing) arranged in a 64x64 configuration, covering an active area of 7.12 mm². *From Maccione et al., J Physiol 2014*

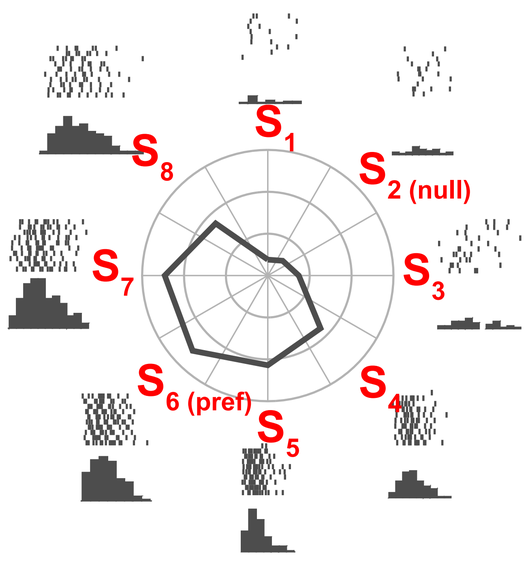


Raw signals recorded from the same channel in control and ALLYL 8hrs condition

Full-array recordings were performed at a sampling frequency of 7.06 kHz. For light stimulation (mean luminance 1.73 µW/cm²) we used a custom built high-resolution photostimulation system based on a DLP video projector (Texas Instruments, USA). We used diffuse full field stimulation (0.5 Hz, 30 trials), square wave gratings (2200 µm half cycle, 960 µm/sec, 8 directions, 5 different contrasts, 10 trials) and a checkerboard stimulus where blocks are shifted randomly in space at fixed time steps (Pamplona et al, CNS 2015).



We estimated each unit's instantaneous firing rate by convolving its spike train with a Gaussian (standard deviation = 25ms). We then computed a Bias Index (Carcieri et al., J.Neurophysiol 2003) that measures the relative amplitude of the ON and the OFF responses.



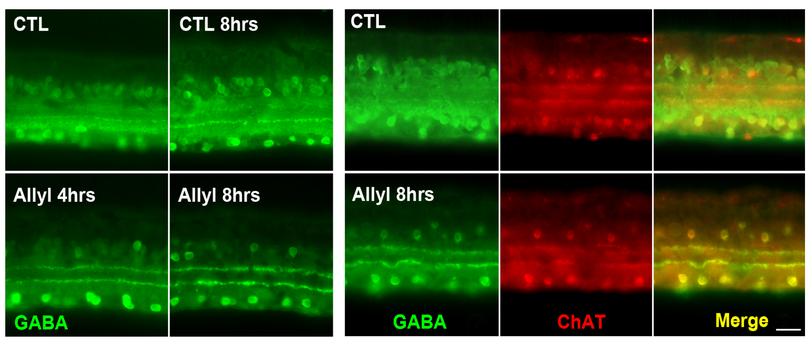
$$DSI = \frac{S_{(pref)} - S_{(null)}}{S_{(pref)} + S_{(null)}} \qquad x^2 = \sum_{i=8}^{8} \frac{(S_i - \bar{S})^2}{\bar{S}}$$

Equations to estimate direction selectivity (DSI) and to quantify anisotropy (χ^2) *(Sernagor and Grzywacz, J Neurophysiol 1995)*.

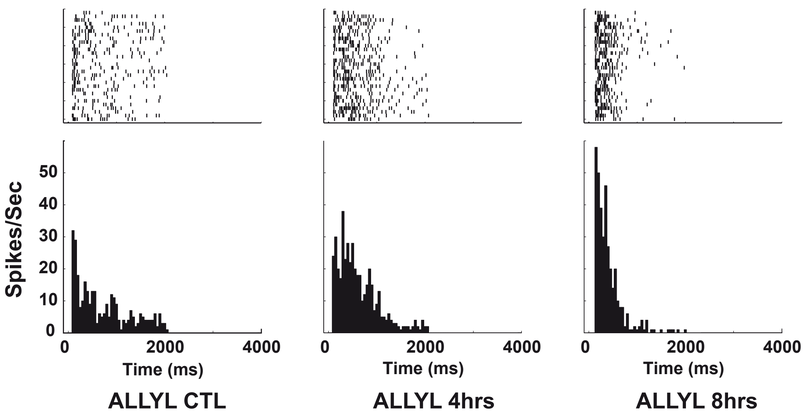
Number of units for statistics

	GABA Block	ALLYL	CTL
Contrast ON RGCs	638	804	
Contrast OFF RGCs	387	528	
DSI ON	228	258	92
CHI2 ALL	4180	5754	3975
RF Diameter	478	869	

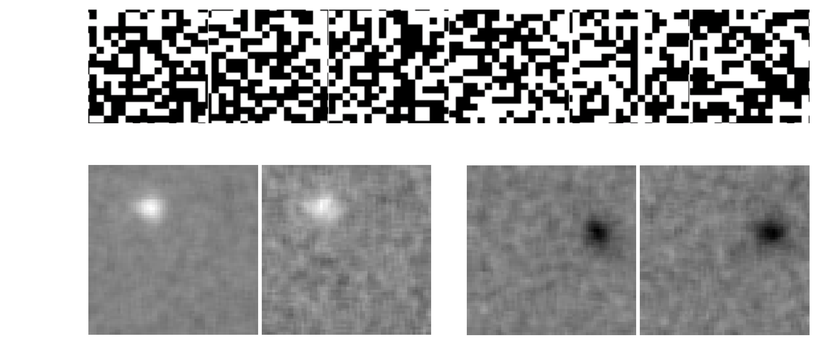
Results

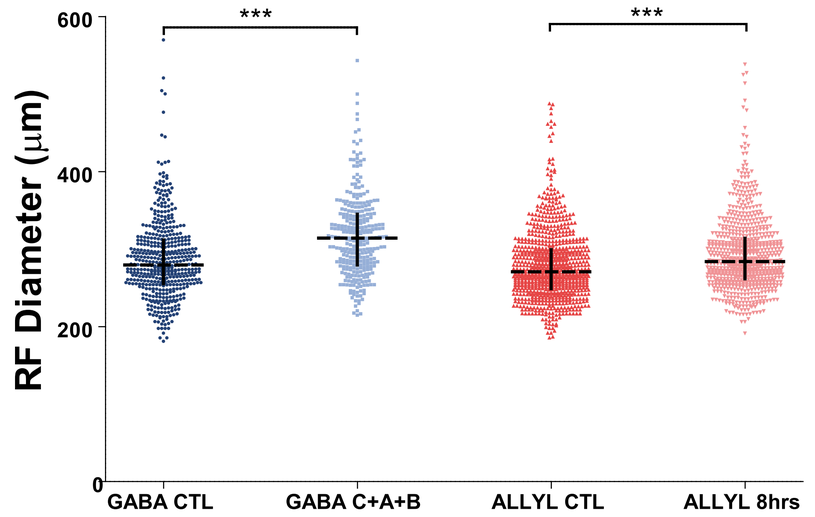


Time course of GABA (green) expression at the onset of the experiment (top left) and up to 8 hours later in normal aCSF. After 8 hours in ALLYL (bottom), the only remaining cells still expressing GABA are the Starburst amacrine cells, co-expressing ChAT (red).

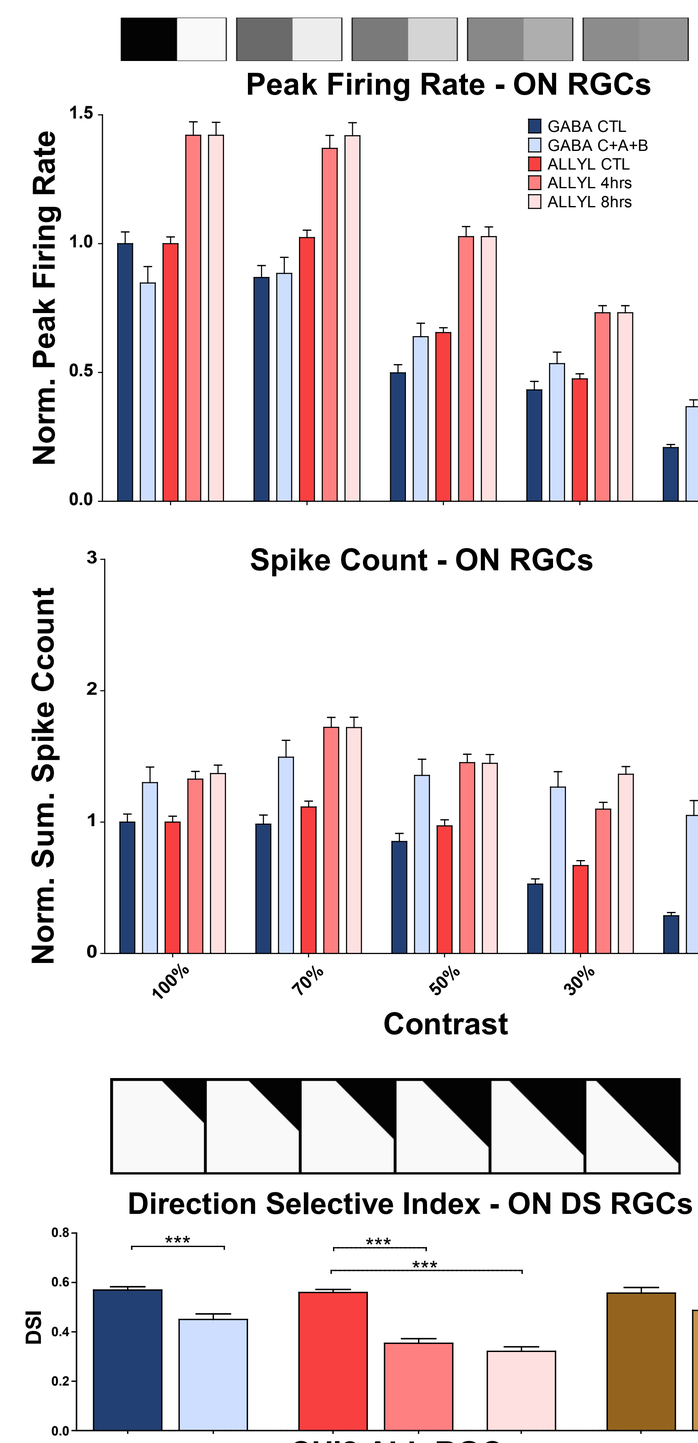


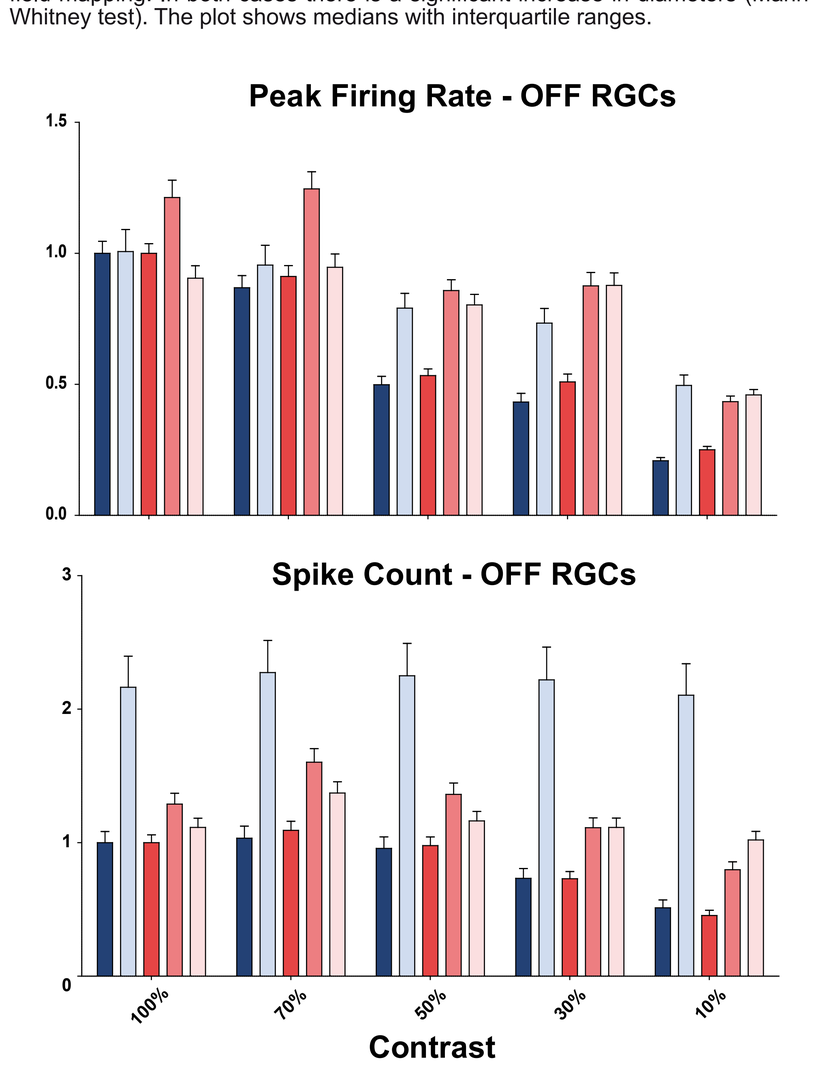
Responses to full field illumination for an ON RGC. Peak firing rates are higher but the duration is shorter for ALLYL 8hrs condition



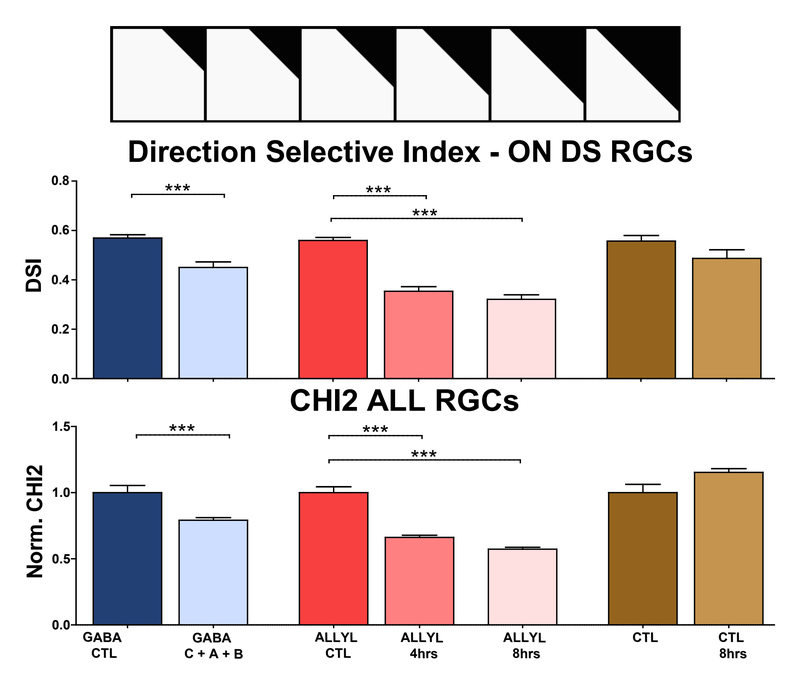


Effect of GABA depletion on responses to shifted white noise stimuli for receptive field mapping. In both cases there is a significant increase in diameters (Mann Whitney test). The plot shows medians with interquartile ranges.





Effect of GABA receptor blockade (blue) and GABA depletion (red) on contrast sensitivity. Bars represent mean with standard errors. Responses for each contrast conditions were pooled together for the different directions of motion.



Effect of GABA receptor blockade (blue) and GABA depletion (red) on DSI distribution in ON DS RGCs (top) and the overall anisotropy, defined by CHI2, of all RGCs (bottom). No significant change was observed when retinas are maintained for 8 hours in normal aCSF (brown). Bars show mean and standard errors of the mean.1way ANOVA (Kruskal Wallis) with Dunn multiple comparison test

Summary

Pharmacological depletion of endogenous retinal GABA reveals interesting changes in

- **motion sensitivity**
- **contrast sensitivity**
- **receptive field properties**

and suggest that in addition to activating its three classical types of receptors, GABA may have some additional trophic roles that influence how RGCs respond to light.

These preliminary experiments provide useful insights into the role of synaptic inhibition in visual processing and this subject deserves further investigation.

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